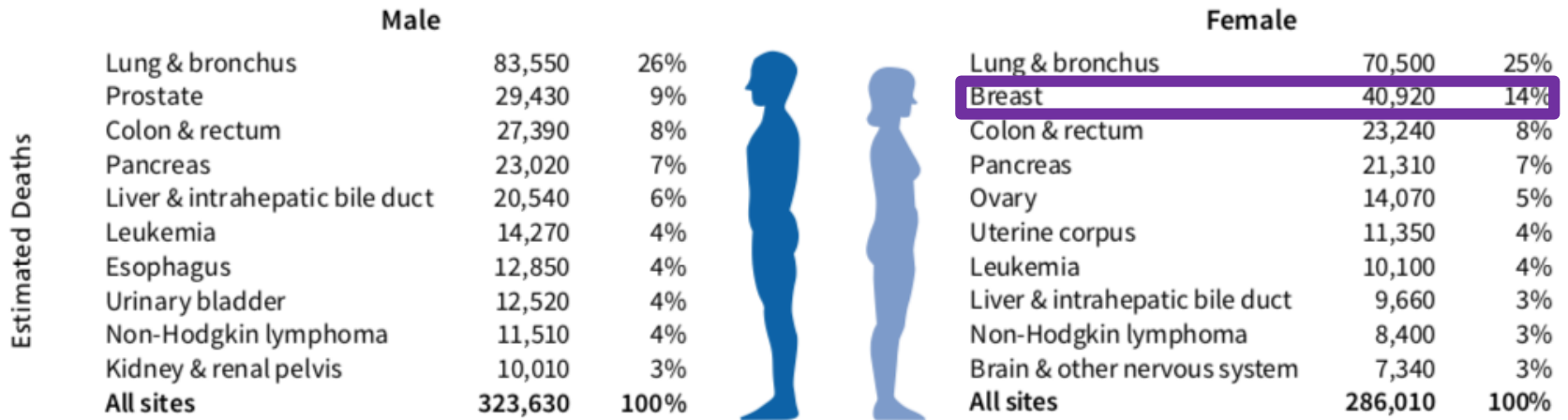# From Tumor to Niche and Back: A materials-based approach to disease-in-a-dish

Jesse Placone, PhD
Biomedical Engineering Program
Department of Physics and Engineering

6th February 2020
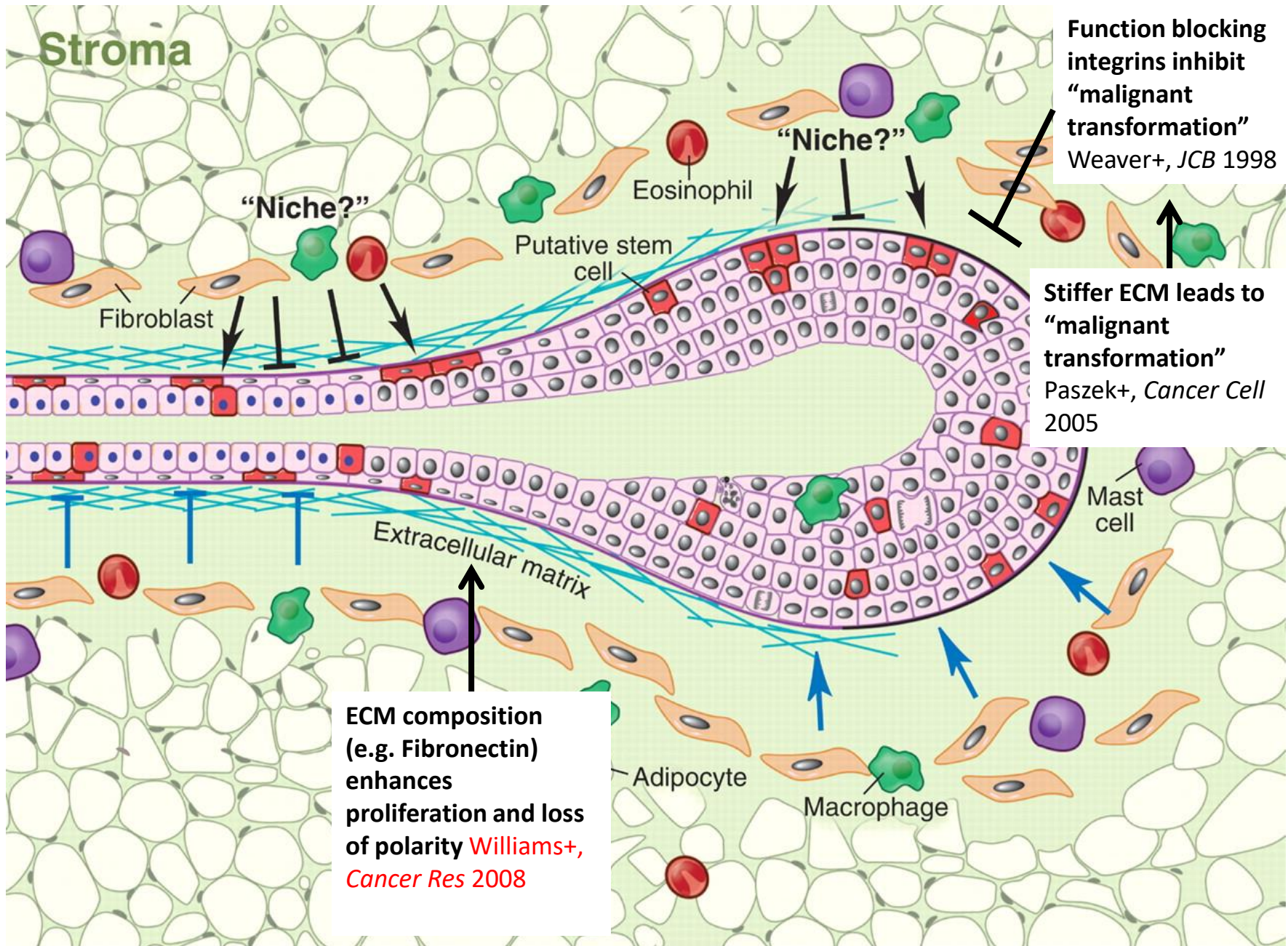West Chester University

Breast cancer is the second leading cause of cancer-related deaths in women

Male

Lung & bronchus	83,550	26%
Prostate	29,430	9%
Colon & rectum	27,390	8%
Pancreas	23,020	7%
Liver & intrahepatic bile duct	20,540	6%
Leukemia	14,270	4%
Esophagus	12,850	4%
Urinary bladder	12,520	4%
Non-Hodgkin lymphoma	11,510	4%
Kidney & renal pelvis	10,010	3%
All sites	**323,630**	**100%**

Female

|		
Lung & bronchus	70,500	25%
Breast	40,920	14%
Colon & rectum	23,240	8%
Pancreas	21,310	7%
Ovary	14,070	5%
Uterine corpus	11,350	4%
Leukemia	10,100	4%
Liver & intrahepatic bile duct	9,660	3%
Non-Hodgkin lymphoma	8,400	3%
Brain & other nervous system	7,340	3%
All sites	**286,010**	**100%**

Estimated Deaths

Estimates are rounded to the nearest 10, and cases exclude basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder. Ranking is based on modeled projections and may differ from the most recent observed data.

©2018, American Cancer Society, Inc., Surveillance Research

L., S. R.; D., M. K.; Ahmedin, J., *CA: A Cancer Journal for Clinicians* (2018)

Stroma

"Niche?"

Fibroblast

Eosinophil

Putative stem cell

"Niche?"

Function blocking integrins inhibit "malignant transformation" Weaver+, *JCB* 1998

Stiffer ECM leads to "malignant transformation" Paszek+, *Cancer Cell* 2005

Mast cell

Extracellular matrix

ECM composition (e.g. Fibronectin) enhances proliferation and loss of polarity Williams+, *Cancer Res* 2008

Adipocyte

Macrophage

Lu and Werb *Science* (2008)

3

Is there a better model for cancer progression given the dynamic nature?

- What happens if acini are formed prior to stiffening of the ECM?

- Can we recapitulate this with an in vitro system?
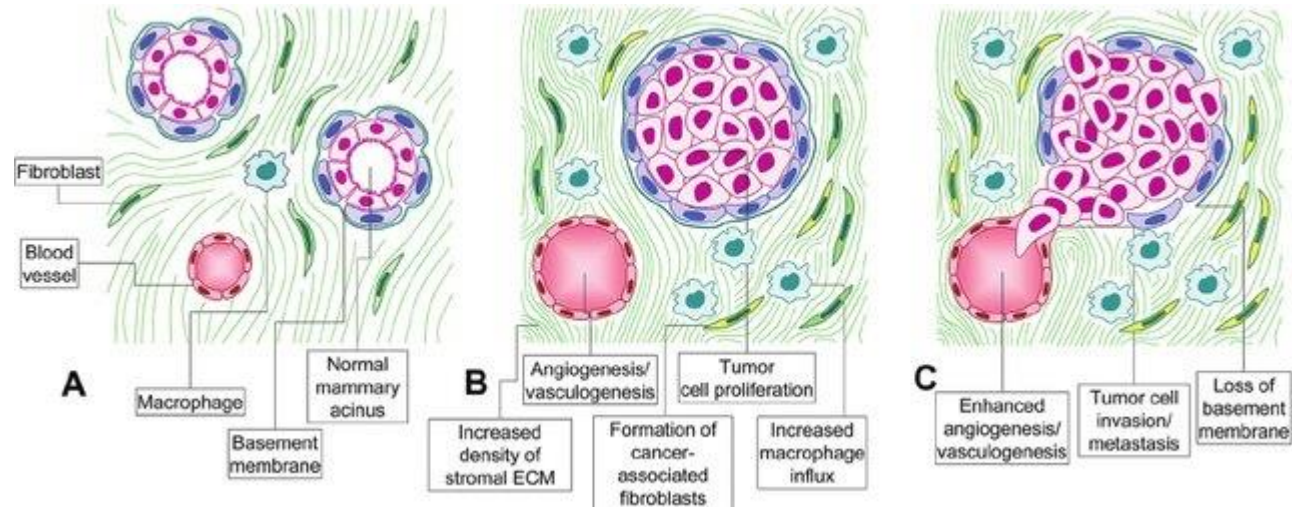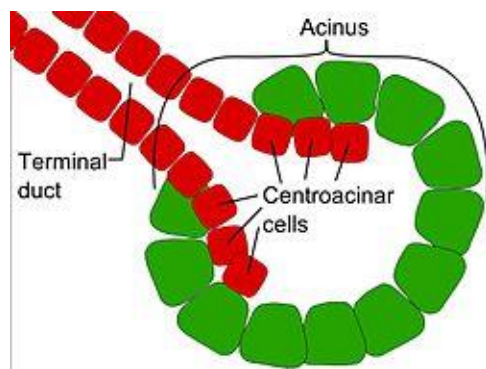
Wikimedia commons

Radisky et al, *Frontiers in Bioscience*, 2015

Dynamic ECM can better mimic acinir niche
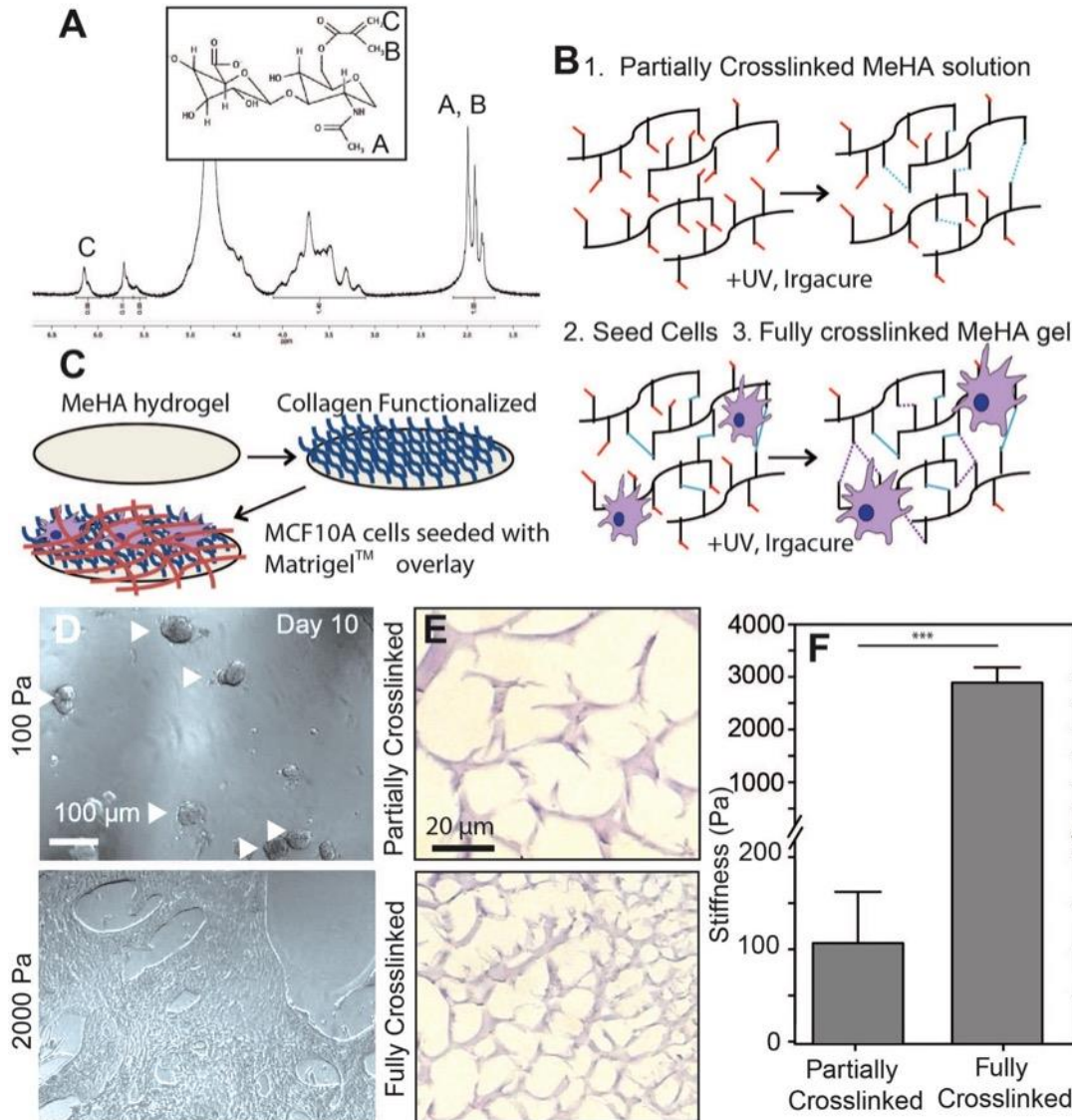
- Stiffness is proportional to UV exposure

- Cells can be seeded on soft or stiff substrates with the same chemistry

Ondeck*, Kumar*, Placone*, et al *PNAS* 2019

Stiffening post-morphogenesis changes the onset of EMT

~Normal mammary gland

~Average Tumor
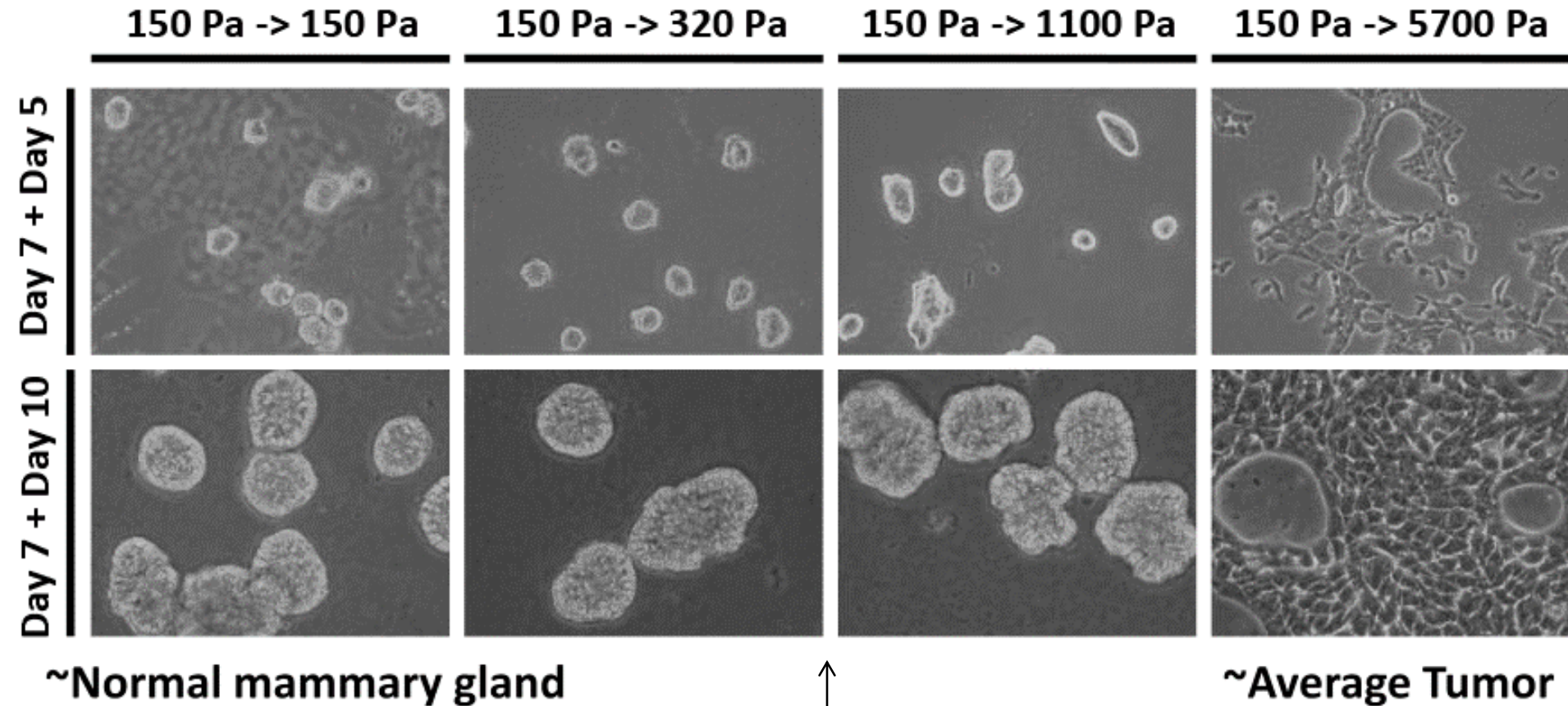
Paszek+ saw change at 450-600 Pa

SMAD and Twist localization is mixed for dynamically stiffened
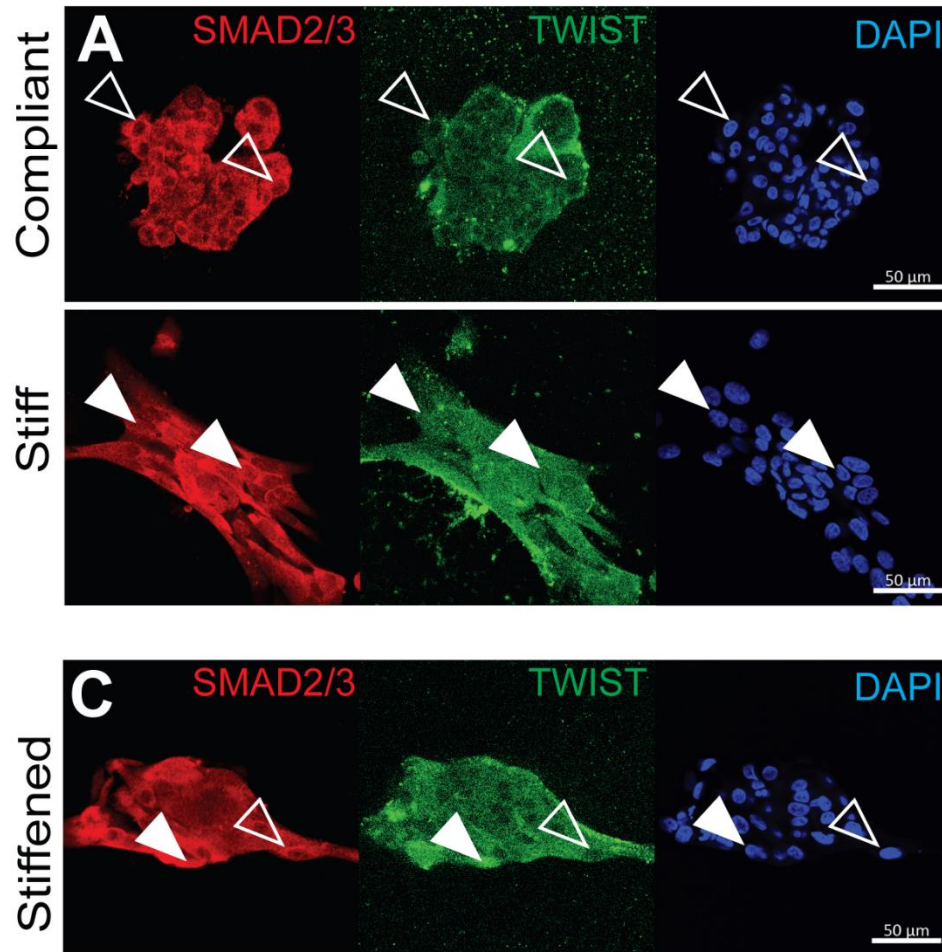
- SMAD and Twist are nuclear localized for stiff hydrogels

- Dynamically stiffened do not have a clear distinction between acini and spread cells

# Inhibition of TGFR modulates SMAD localization and decreases response to stiffening
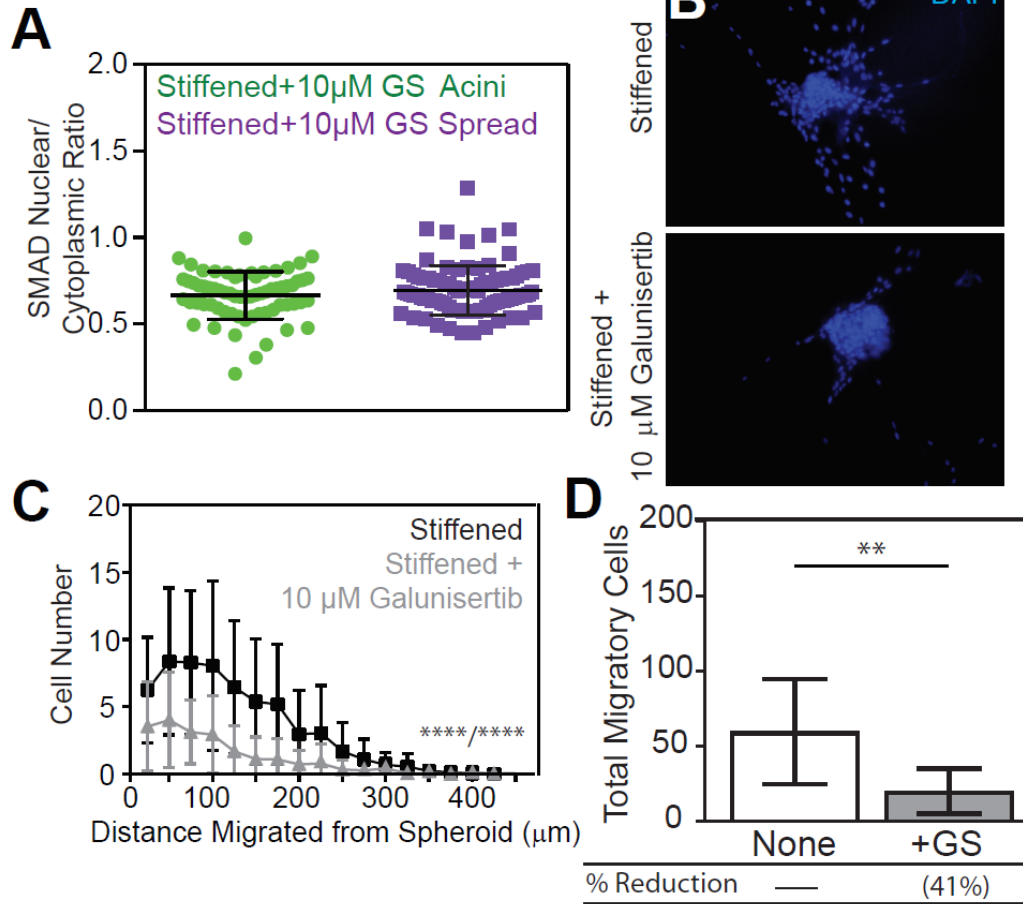
- Galunisertib inhibits TGFR kinase activity

- SMAD2/3 is downstream of TGFR

- Inhibition reduces total number of migratory cells even after stiffening

Ondeck*, Kumar*, Placone*, et al *PNAS* 2019

SMAD and YAP localization is mixed for dynamically stiffened
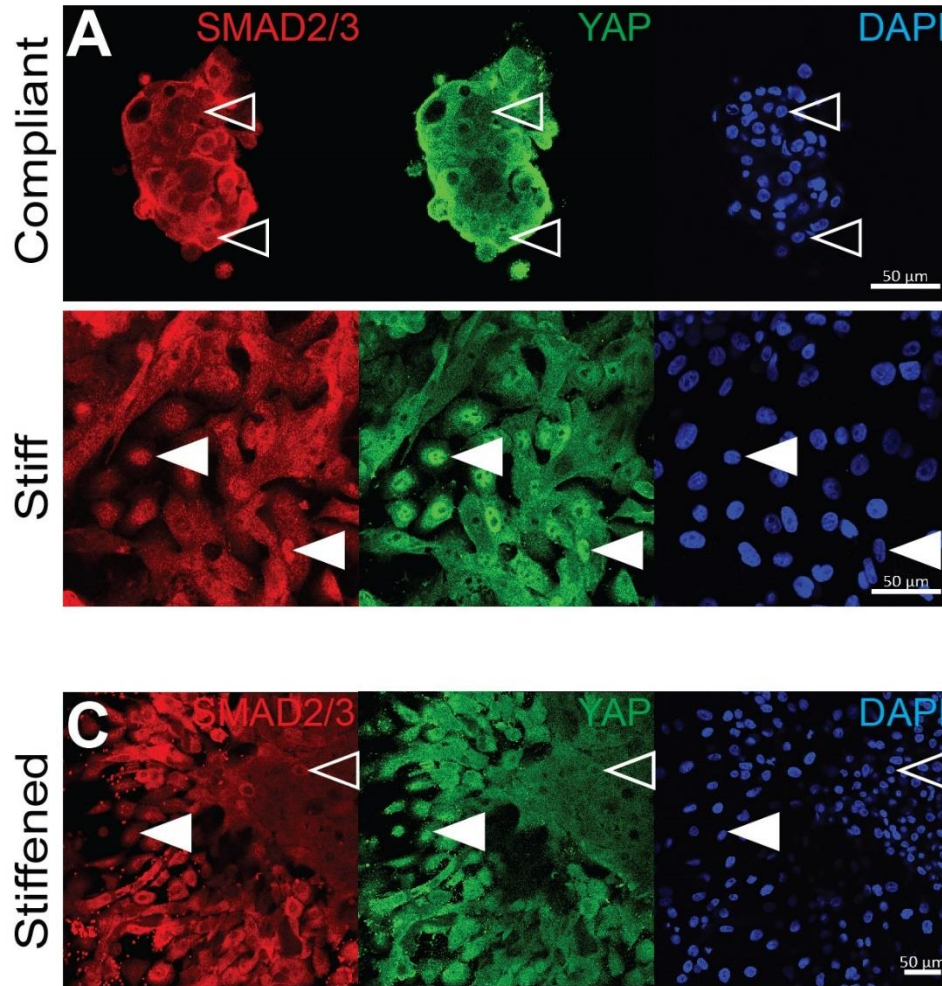
- SMAD and YAP are nuclear localized for stiff hydrogels

- YAP nuclear localization tends to be a better indicator for spreading than SMAD alone

Inhibition of YAP activity and expression inhibits EMT response to stiffening
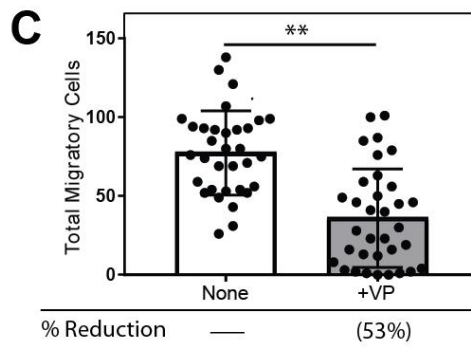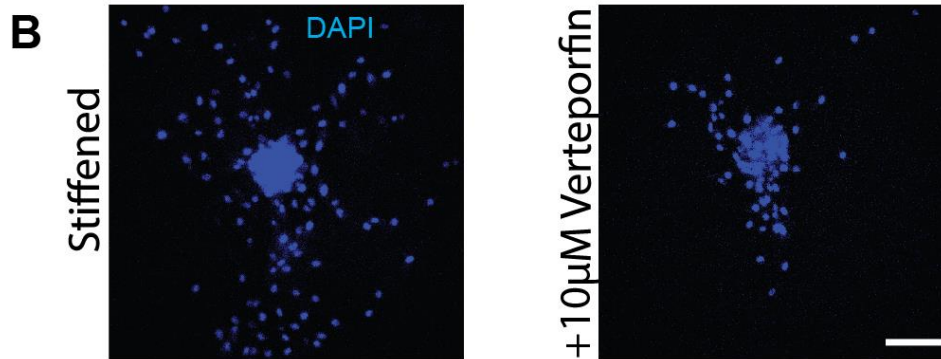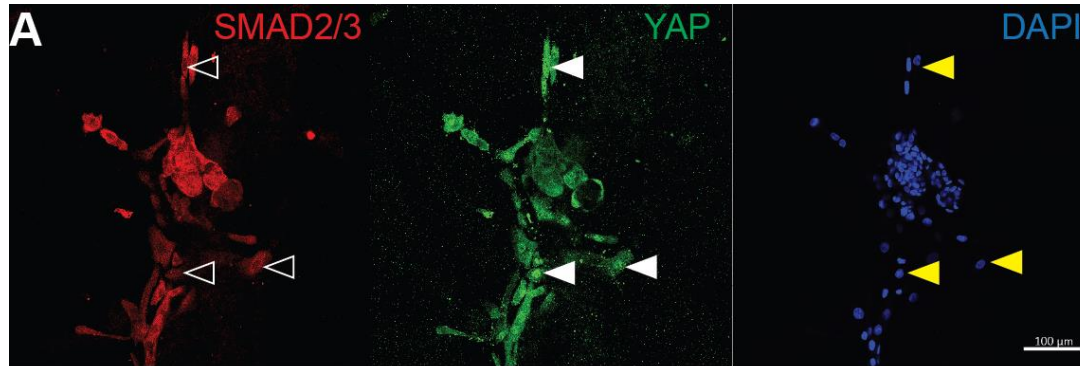
- Verteporfin inhibits YAP activity and expression (not localization)

- Inhibition reduces total number of migratory cells even after stiffening

Ondeck*, Kumar*, Placone*, et al *PNAS* 2019

Dual inhibition of cytokine and mechanical signaling significantly reduces EMT response
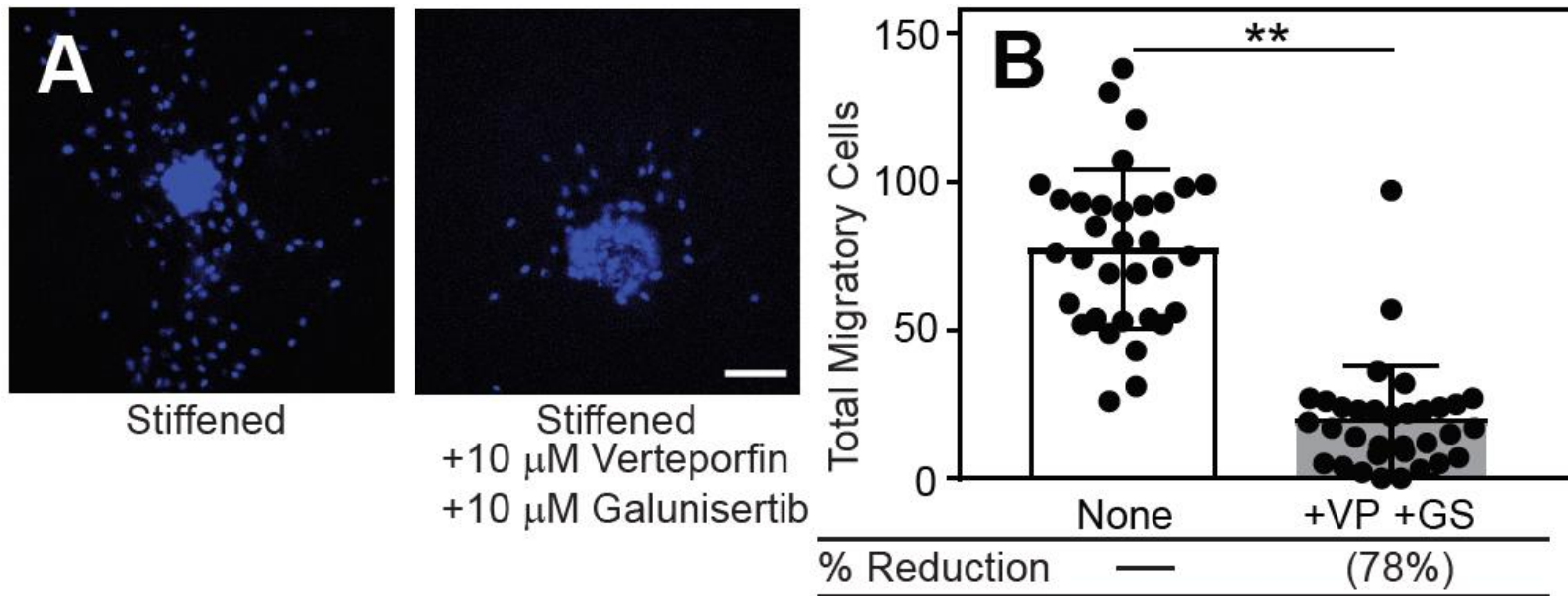
- Galunisertib and Verteporfin significantly reduce total number of migratory cells

- Number of cells leaving is decreased when compared to GS alone (41%) and VP alone (53%)

# Dynamic models elucidate interplay between different EMT pathways

- Mammary tissue stiffens over time
  - Changes threshold of stiffness-sensitive EMT
- Inhibition of TGFR and YAP reduces spreading
  - TGFR inhibitor alone only partially reduces EMT response due to stiffening
  - YAP inhibition only prevents a subpopulation from responding
  - Dual inhibition reduces ~78% of cell response to stiffening

Cancer metastasis responsible for high mortality
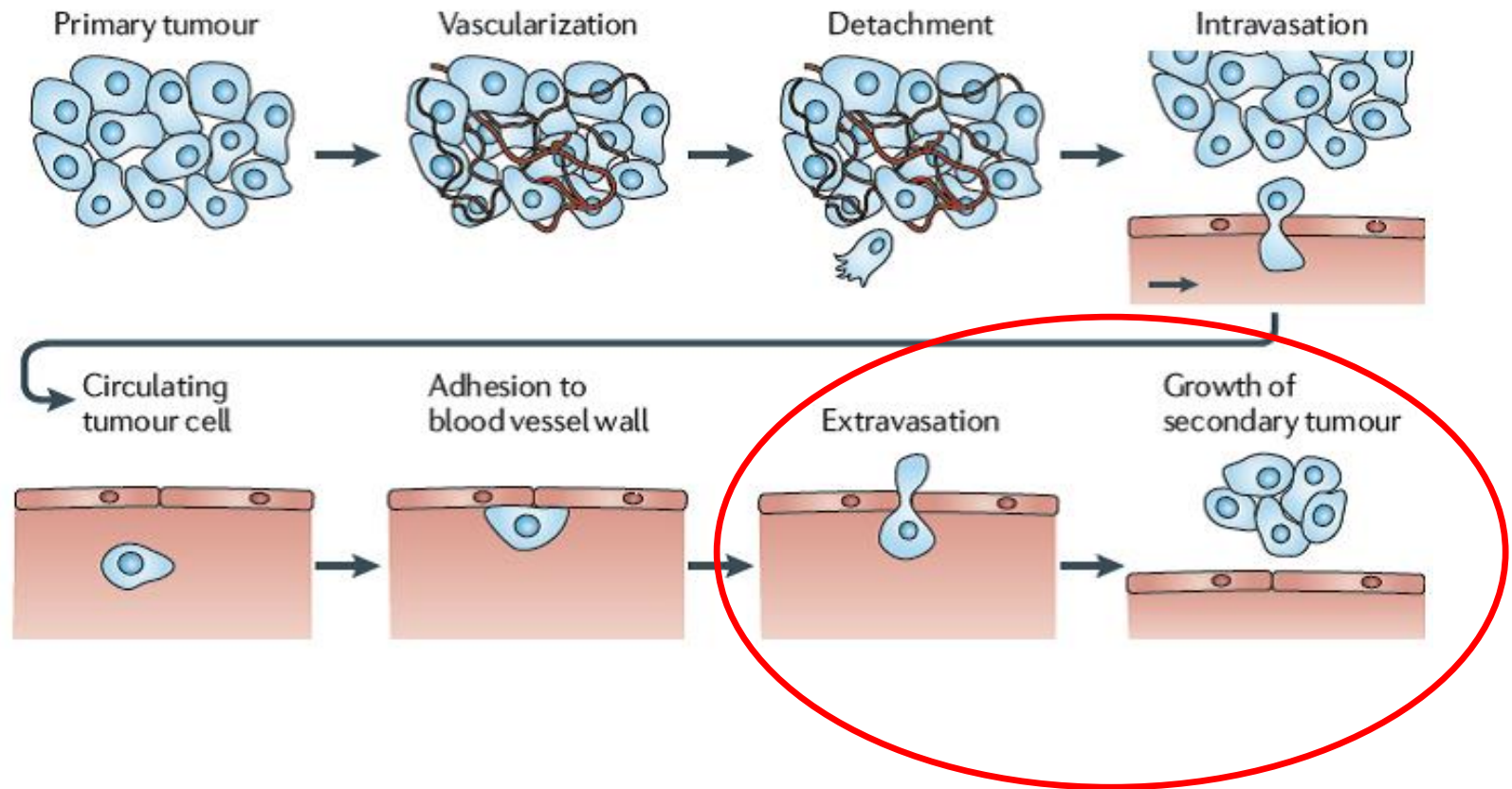
Vasculature in cancer metastasis

- In vivo tumor tissue is highly vascularized

- Interactions with blood vessels are key for understanding metastasis

Servier Medical Art; https://smart.servier.com/
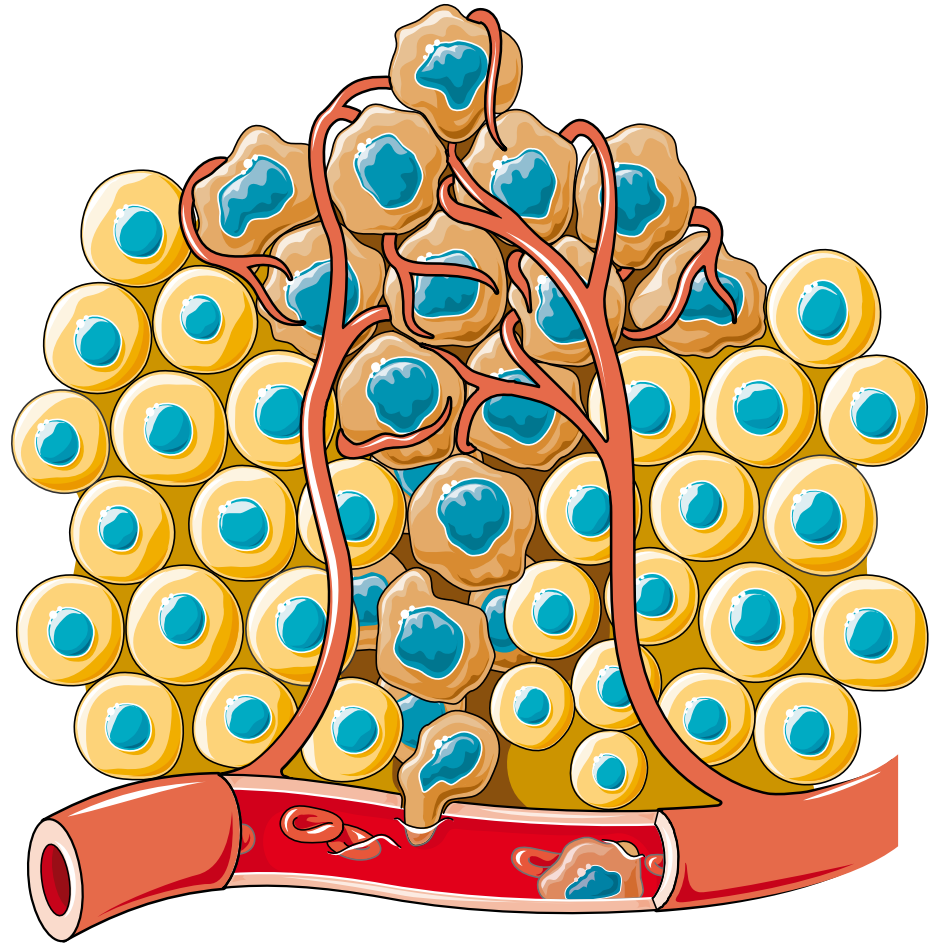
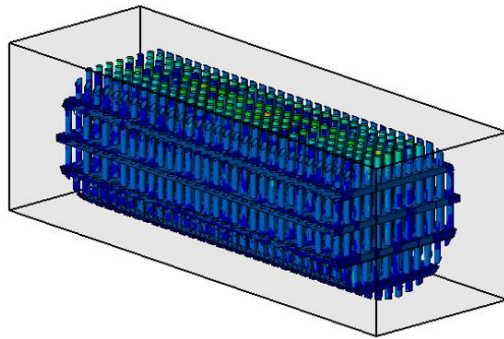# Can we develop a simplified model of this process using additive manufacturing?

YES!

We will need:

- A bone mimetic

- A vascular mimetic

- And the cell types present in each

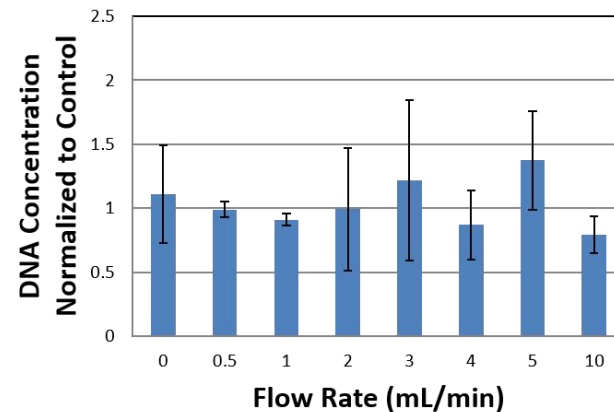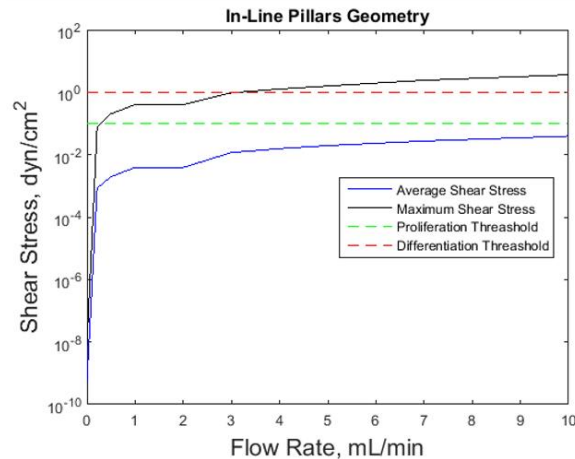# Bioreactors can recapitulate physiological environment of bone

- Dynamic culture system can enhance proliferation of adherent and non-adherent cell types (e.g. MSCs and HSPCs)
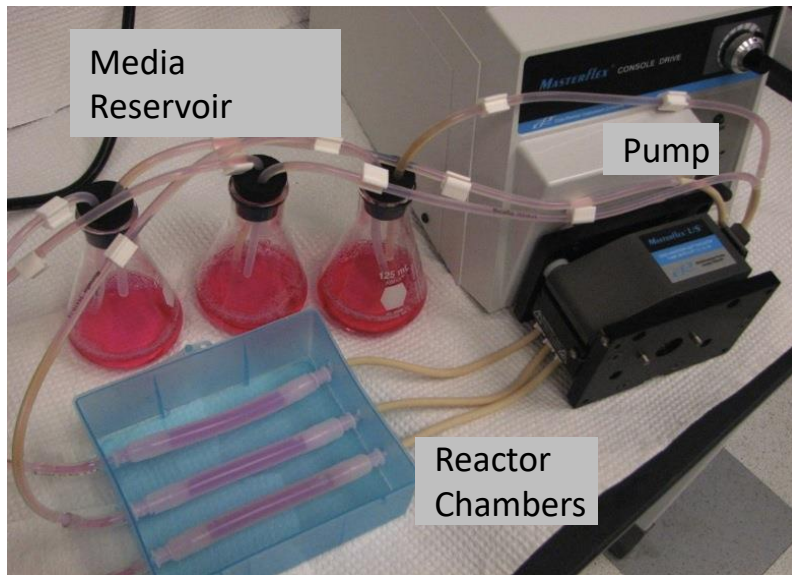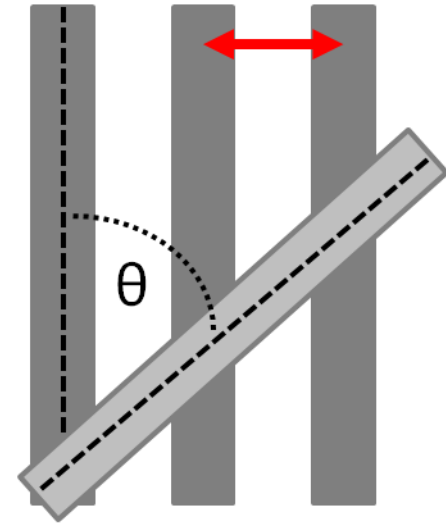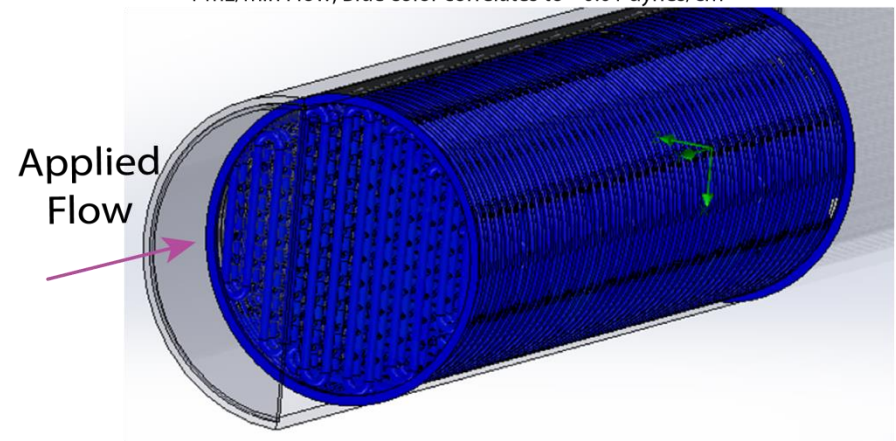

Shear Stress
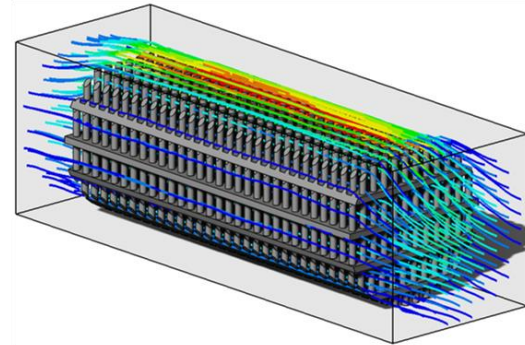
Fluid Velocity

Fluid simulations allow for design libraries to be tested in silico
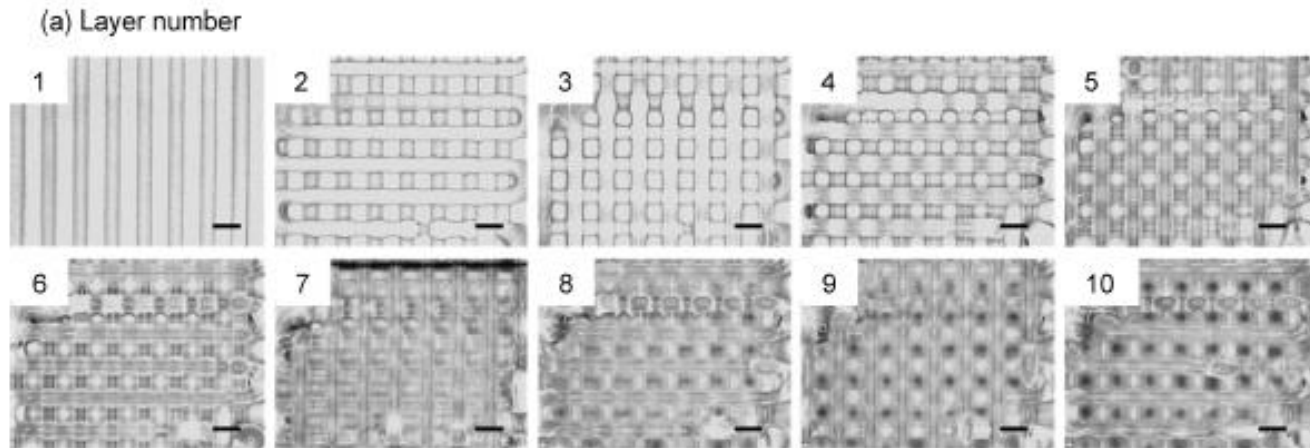
- Generate flow simulation libraries

  - Vary parameters *in silico*

  - Filament size

  - Offset angle

  - Filament spacing

- Reduces number of *in vitro* & *vivo* samples

Media Reservoir

Pump

Reactor Chambers

Computational Fluid Dynamics Model:
1 mL/min Flow; Blue color correlates to ~0.01 dynes/cm²

Applied Flow

AB Yeatts and JP Fisher. Tissue Engineering Part C, Methods. (2011).

Factorial design allows for generation of prints with tightly controlled geometry

Trachtenberg, Placone, et al. *ACS Biomater. Sci. Eng.* 2016
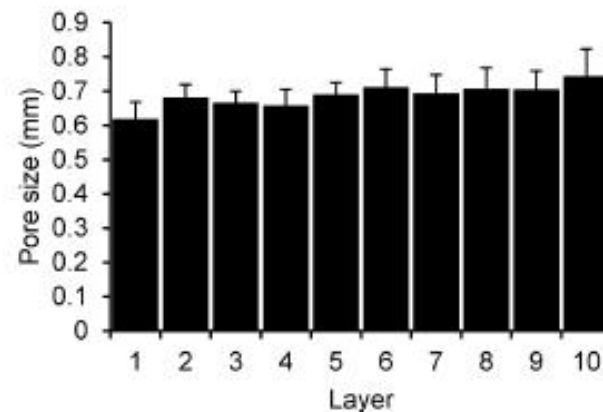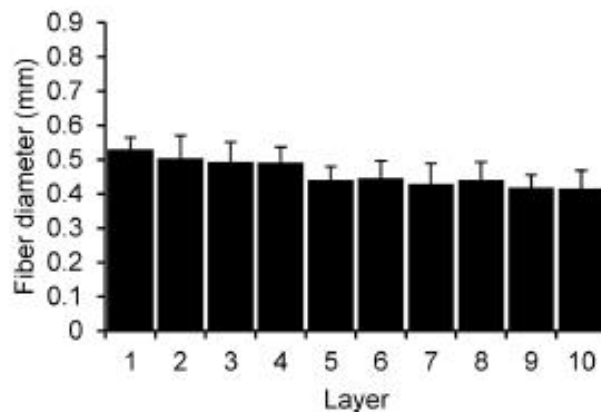
Pore spacing and subsequently shear stress can be tailored layer-by-layer

Trachtenberg JE, et al *ACS Biomaterials Science & Engineering*. 2017
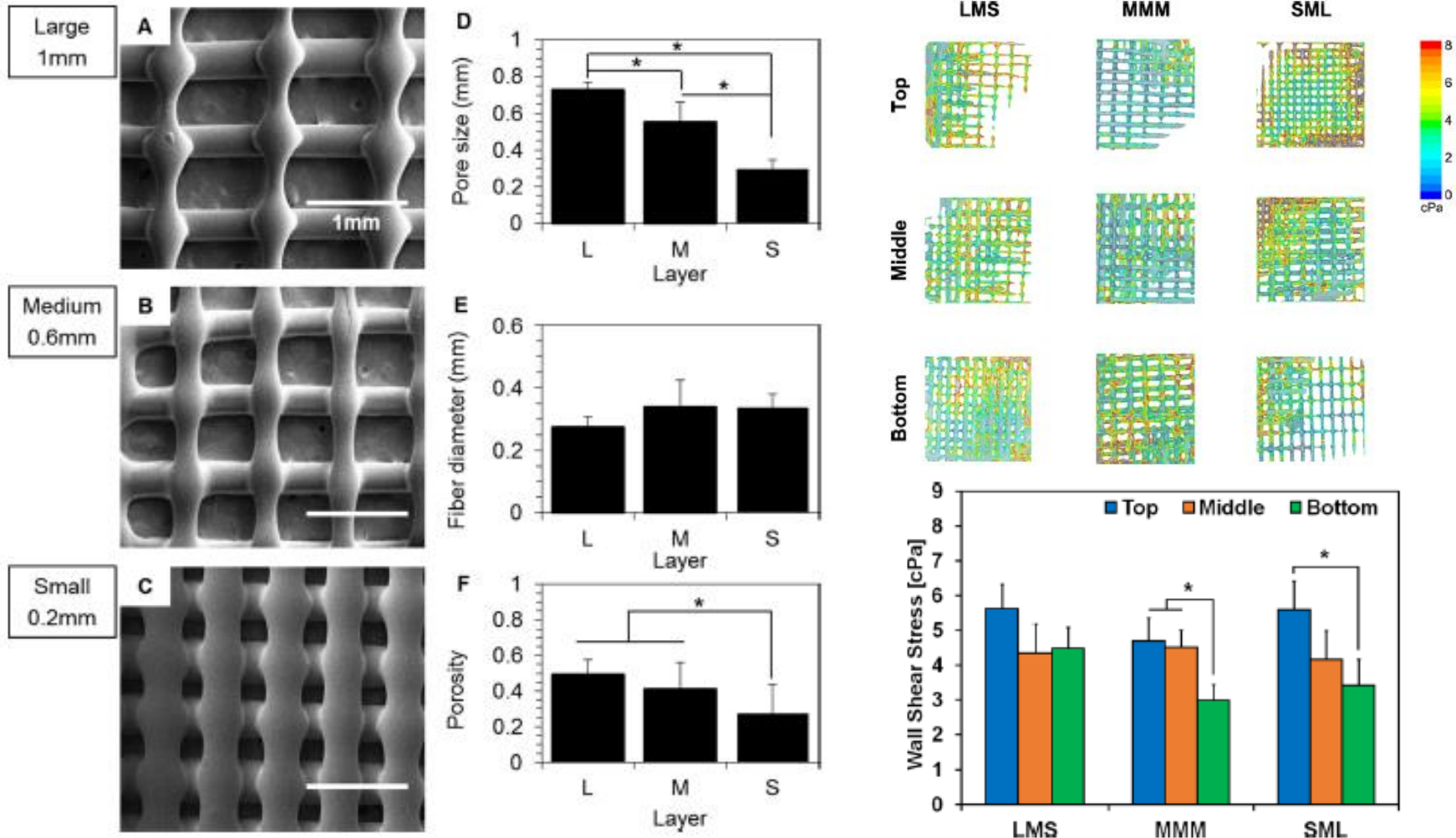
Additive manufacturing lends itself to being an excellent way to develop bone mimetics

- Multiple materials have been developed for bone mimetics

- Pore sizes and spaces can be tuned to obtain desired shear stress and nutrient diffusion profiles

- MSCs and non-adherent cells can be cultured on these scaffolds

- These can be directly translated to a bone niche microenvironment

# Project 1: Towards GMP-compliant MSC and osteoblast production

Aims:

- Model in silico shear stresses and nutrient diffusion

- Characterize MSC response to substrate geometries

- Demonstrate stem-like behavior by ability to generate 3 main lineages

- Statistical characterization of print fidelity
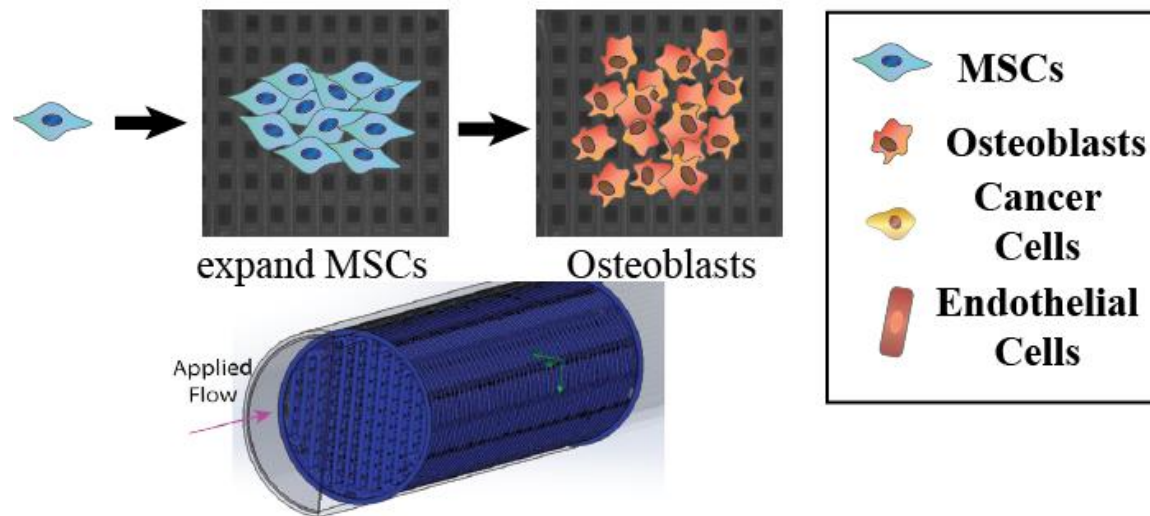
Project 2: 3D microenvironments to understand mammary to bone (M2B) metastasis and signaling

Aims:

- Quantify invasion as a function of circulating cancer cell density, time, and preconditioned status

- Histological and immunofluoresence characterization

- Circulating cancer cell survival and attachment assays

- Temporal assessment of invasion

Circulating cancer cells → Homing to 3DP substrate
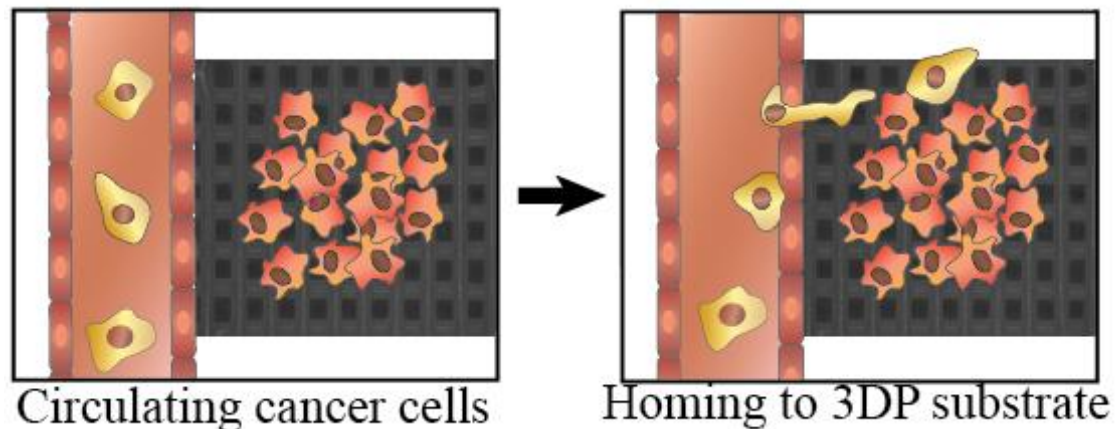
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